

WHAT IS CLAIMED IS:

- 1 1. A method of refolding a first insoluble, recombinant, eukaryotic
2 glycosyltransferase, wherein the glycosyltransferase comprises a maltose binding protein
3 domain (MBD), the method comprising the steps of
4 (a) solubilizing the insoluble, recombinant, eukaryotic glycosyltransferase in a
5 solubilization buffer; and
6 (b) contacting the soluble eukaryotic glycosyltransferase with a refolding
7 buffer comprising a redox couple to refold the eukaryotic glycosyltransferase, wherein the
8 refolded eukaryotic glycosyltransferase catalyzes the transfer of a sugar from a donor
9 substrate to an acceptor substrate.
- 1 2. The method of claim 1, wherein the first eukaryotic glycosyltransferase
2 is truncated to remove all or a portion of a stem region.
- 1 3. The method of claim 1, wherein an unpaired cysteine in the first
2 eukaryotic glycosyltransferase is removed by substitution with a non-cysteine amino acid.
- 1 4. The method of claim 1, wherein the first eukaryotic glycosyltransferase
2 is selected from the group consisting of GnT1, GalT1, StIII Gal3, St3GalI, St6 GalNAcT1,
3 Core GalIT1, GalNAcT2.
- 1 5. The method of claim 1, wherein the first eukaryotic glycosyltransferase
2 further comprises a purification domain selected from the group consisting of a starch
3 binding domain, a thioredoxin domain, a SUMO domain, a poly-His domain, a myc epitope
4 domain, and a glutathione-S-transferase domain.
- 1 6. The method of claim 1, wherein the first eukaryotic glycosyltransferase
2 further comprises a self cleaving domain.
- 1 7. The method of claim 1, wherein the first eukaryotic glycosyltransferase
2 is expressed in a bacterial host cell as an insoluble inclusion body.
- 1 8. The method of claim 1, wherein a second insoluble, recombinant
2 eukaryotic glycosyltransferase is refolded with the first eukaryotic glycosyltransferase.

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1 9. The method of claim 8, wherein a third insoluble, recombinant
2 eukaryotic glycosyltransferase is refolded with the first eukaryotic glycosyltransferase and
3 the second eukaryotic glycosyltransferase.

1 10. The method of claim 1, wherein the redox couple is selected from the
2 group consisting of reduced glutathione/oxidized glutathione (GSH/GSSG) and cysteine/
3 cystamine.

1 11. The method of claim 1, wherein the acceptor substrate is selected from
2 a protein, a peptide, a glycoprotein, and a glycopeptide.

1 12. The method of claim 1, wherein the first eukaryotic glycosyltransferase
2 is a sialyltransferase.

1 13. The method of claim 12, wherein the sialyltransferase is selected from
2 the group consisting of StIII Gal3, St3GalI, St6 GalNAcT1.

1 14. The method of claim 12, wherein the donor substrate is a CMP-sialic
2 acid PEG molecule and the acceptor substrate is selected from a protein, a peptide, a
3 glycoprotein, and a glycopeptide.

1 15. A recombinant, eukaryotic glycosyltransferase, wherein a stem anchor
2 region and a transmembrane domain are deleted from the recombinant, eukaryotic
3 glycosyltransferase, and wherein the glycosyltransferase is fused in frame to a maltose
4 binding domain.

1 16. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 all or a portion of the stem region is deleted.

1 17. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 an unpaired cysteine in the recombinant, eukaryotic glycosyltransferase is removed by
3 substitution with a non-cysteine amino acid.

1 18. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is selected from the group consisting of a GnT1 protein, a GalT1
3 protein, an StIII Gal3 protein, an St3GalI protein, an St6 GalNAcT1 protein, a Core GalIT1
4 protein, and a GalNAcT2 protein.

1 19. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is a GnT1 protein.

1 20. The GnT1 protein of claim 19, wherein the GnT1 protein is a truncated
2 human GnT1 protein selected from GnT1 Δ 35 and GnT1 Δ 103.

1 21. The GnT1 protein of claim 19, wherein the GnT1 protein is a human
2 GnT1 protein comprising an unpaired cysteine substitution selected from the group consisting
3 of CYS121ALA, CYS121ASP, and ARG120ALA, CYS121HIS.

1 22. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is a GalT1 protein.

1 23. The GalT1 protein of claim 22, wherein the GalT1 protein is a
2 truncated bovine GalT1 protein selected from GalT1 Δ 70 and GalT1 Δ 129.

1 24. The GalT1 protein of claim 22, wherein the GalT1 protein is a bovine
2 GalT1 protein comprising an unpaired cysteine substitution of CYS342THR.

1 25. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is an ST3GalIII protein.

1 26. The ST3GalIII protein of claim 25, wherein the ST3GalIII protein is a
2 truncated rat ST3GalIII protein selected from ST3GalIII Δ 28, ST3GalIII Δ 73, ST3GalIII Δ 85
3 and ST3GalIII Δ 86.

1 27. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is a Core1 GalT1 protein.

1 28. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is an ST3Gal1 protein.

1 29. The ST3Gal1 protein of claim 28, wherein the ST3Gal1 protein is a
2 truncated human ST3Gal1 protein selected from ST3Gal1 Δ 29, ST3Gal1 Δ 45, and ST3Gal1
3 Δ 56.

1 30. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is an ST6GalNAc1 protein.

1 31. The recombinant, eukaryotic glycosyltransferase of claim 15, wherein
2 the glycosyltransferase is an GalNAcT2 protein.

1 32. The GalNAcT2 protein of claim 31, wherein the GalNAcT2 protein is
2 a truncated human GalNAcT2 protein selected from GalNAcT2 Δ 40, GalNAcT2 Δ 51,
3 GalNAcT2 Δ 74 and GalNAcT2 Δ 95.

1 33. A method of remodeling a protein, a peptide, a glycoprotein, or a
2 glycopeptide using the recombinant, eukaryotic glycosyltransferase of claim 15.

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